

Applicant : Piet Boender
Appl. No. : Not Yet Assigned
Filed : Herewith

Amendments to the Claims:

Please cancel Claims 1-26 without disclaimer or prejudice to applicant's right to pursue the subject matter of these claims in a future divisional or continuation application, and add new Claims 27-50 as set forth below.

1-26 (cancelled).

27. (new) A method for generating multiple RNA copies comprising the steps of:

(a) providing a sample comprising target RNA, wherein said sample is simultaneously contacted with:

- an oligonucleotide comprising at its 5' side a promoter sequence recognized by an RNA polymerase, wherein said oligonucleotide further comprises:
 - a target hybridising sequence, which is a random sequence,
 - a modified nucleotide at its 3' terminal end in such a way that extension therefrom is prohibited, wherein said modified nucleotide is chosen from the group comprising nucleotides comprising alkane-diol residues, cordycepsins, amino-alkyls, and dideoxynucleotides,
 - at least one chimeric linkage between nucleotides at the 3' end; wherein said chimeric linkage may contain at least one phosphorothioate linkage between nucleotides, or a PNA, LNA or GripNA backbone, and;
- an enzyme having DNA polymerase activity;
- an enzyme having RNase H activity;
- an enzyme having RNA polymerase activity; and

Applicant : Piet Boender
Appl. No. : Not Yet Assigned
Filed : Herewith

- sufficient amounts of nucleotides; and
- (b) maintaining the resulting reaction mixture under the appropriate conditions for a sufficient amount of time for the enzymatic processes to take place.

28. (new) A method for generating multiple RNA copies comprising the steps of:

(a) providing a sample comprising target RNA, wherein said sample is simultaneously contacted with:

- a DNA oligonucleotide comprising at its 5' side a promoter sequence recognized by an RNA polymerase, wherein said oligonucleotide further comprises:
 - a target hybridising sequence, which is a predetermined sequence,
 - a modified nucleotide at its 3' terminal end in such a way that extension therefrom is prohibited, wherein said modified nucleotide is chosen from the group comprising nucleotides comprising alkane-diol residues, cordycepins, amino-alkyls, and dideoxynucleotides,
 - at least one chimeric linkage between nucleotides at the 3' end; wherein said chimeric linkage may contain at least one phosphorothioate linkage between nucleotides, or a PNA, LNA or GripNA backbone, and;
- an enzyme having Klenow pol I exo (-) activity;
- an enzyme having RNase H activity;
- an enzyme having RNA polymerase activity; and
- sufficient amounts of nucleotides; and

Applicant : Piet Boender
Appl. No. : Not Yet Assigned
Filed : Herewith

(b) maintaining the resulting reaction mixture under the appropriate conditions for a sufficient amount of time for the enzymatic processes to take place.

29. (new) A method for generating multiple RNA copies comprising the steps of:

(a) providing a sample comprising target RNA; wherein said sample is simultaneously contacted with:

- an oligonucleotide comprising at its 5' side a promoter sequence recognized by an RNA polymerase, wherein said oligonucleotide further comprises:
 - a target hybridising sequence, wherein said hybridising sequence is a predetermined sequence,
 - a modified nucleotide at its 3' terminal end in such a way that extension therefrom is prohibited, wherein said modified nucleotide is chosen from the group comprising nucleotides comprising alkane-diol residues, cordycepins, amino-alkyls, and dideoxynucleotides,
 - at least one chimeric linkage between nucleotides at the 3' end; wherein said chimeric linkage may contain at least one phosphorothioate linkage between nucleotides, or a PNA, LNA or GripNA backbone, and,
- an enzyme having DNA polymerase activity;
- an enzyme having RNase H activity;
- an enzyme having RNA polymerase activity; and
- sufficient amounts of nucleotides; and

(b) maintaining the resulting reaction mixture under the appropriate conditions for a sufficient amount of time for the enzymatic processes to take place.

Applicant : Piet Boender
Appl. No. : Not Yet Assigned
Filed : Herewith

30. (new) The method according to claim 27, wherein said target RNA is of eukaryotic, prokaryotic or viral origin, or a mixture thereof.

31. (new) The method according to claim 27, wherein said target RNA is chosen from the group comprising total RNA, mRNA, cRNA, rRNA, tmRNA, asRNA, hnRNA or tRNA, including any combination thereof.

32. (new) The method according to claim 28, wherein said predetermined sequence is chosen from the group comprising gene-specific sequences, viral sequences, prokaryotic sequences, mutation-specific sequences, poly-T sequences, genomic sequences and rRNA.

33. (new) The method according to claim 27, wherein at least one of the nucleotides, e.g. dNTPs and rNTPs, is provided with a label.

34. (New) The method according to claim 27, wherein the generated RNA is used as input material for further amplification.

35. (new) The method according to claim 27, wherein the generated RNA is contacted with:

- an RNA ligase,
- a double stranded nucleic acid complex comprising a double stranded DNA promoter sequence that can be recognized by an RNA polymerase, whereby one strand of said complex has a stretch of RNA attached to the 5' end of one of the DNA strands,
- an enzyme having RNA polymerase activity, and

Applicant : Piet Boender
Appl. No. : Not Yet Assigned
Filed : Herewith

- sufficient amounts of dNTPs and rNTPs;

wherein the resulting reaction mixture is maintained under the appropriate conditions for a sufficient amount of time for the enzymatic processes to take place.

36. (new) The method according to claim 27, wherein the reaction mixture further comprises:

- an RNA ligase; and
- a double stranded nucleic acid complex comprising a double stranded DNA promoter sequence that can be recognized by the RNA polymerase, whereby one strand of said complex has a stretch of RNA attached to the 5' end of one of the DNA strands.

37. (new) The method according to claim 27, wherein the generated RNA copies are contacted with poly A polymerase.

38. (new) The method according to claim 27, wherein the starting material is simultaneously contacted with a poly A polymerase.

39. (new) The method according to claim 27, wherein said promoter sequence is a T7 promoter sequence.

40. (new) The method according to claim 27, wherein said RNA polymerase is a T7 RNA polymerase.

41. (new) The method according to claim 27, wherein said enzyme having DNA polymerase activity is AMV-RT or MMLV-RT.

Applicant : Piet Boender
Appl. No. : Not Yet Assigned
Filed : Herewith

42. (new) The method according to claim 27, wherein said enzyme having RNase H activity is *E. coli* RNase H .

43. (new) The method according to claim 27, wherein said enzyme having RNase H activity is reverse transcriptase.

44. (new) The method according to claim 43, wherein said enzyme having RNase H activity is AMV-RT or MMLV-RT.

45. (new) A method for determining differences in gene expression in cell samples, comprising the steps of:

- creating multiple RNA copies of one or more target RNA species according to the method of claim 27, whereby a first pattern of expression is formed from the sample;
- comparing said first pattern of expression with a predetermined pattern of expression, whereby differences in gene expression are determined.

46. (new) The method according to claim 27, wherein said multiple RNA copies are used to interrogate a probe array.

47. (new) The method according to claim 46, wherein said probe array is an oligonucleotide array.

48. (new) Kit for generating multiple RNA copies comprising:
an oligonucleotide comprising at its 5' side a promoter sequence recognized by an RNA polymerase, wherein said oligonucleotide further comprises a

Applicant : Piet Boender
Appl. No. : Not Yet Assigned
Filed : Herewith

target hybridising sequence, which is a random sequence or a predetermined sequence, said predetermined sequence complexing predominantly with the intended target RNA to be amplified, a modification at its 3' terminal end in such a way that extension therefrom is prohibited, and at least one chimeric linkage between nucleotides at the 3' end, wherein said chimeric linkage may contain at least one phosphorothioate linkage between nucleotides, or PNA, LNA or GripNA backbone, and

- instructions to carry out the method according to claim 27 for generating multiple RNA copies.

49. (new) The kit according to claim 48, further comprising:

- an RNA ligase,
- a double stranded nucleic acid complex comprising a double stranded DNA promoter sequence that can be recognized by an RNA polymerase, whereby one strand of said complex has a stretch of RNA attached to the 5' end of one of the DNA strands, and
- instructions to carry out further amplification.

50. (new) The kit according to claim 48, further comprising a probe array.